

# Cytological mechanisms of $2n$ pollen formation in the wild potato *Solanum okadae* and pollen-pistil relations with the cultivated potato, *Solanum tuberosum*

Elsa Lucila Camadro · Sandra Karina Saffarano · Juan Carlos Espinillo ·  
Mateo Castro · Phillip W. Simon

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**Abstract** *Solanum okadae* Hawkes et Hjert is a wild diploid potato species endemic to Argentina and Bolivia, of potential breeding value. However, no genetic studies have been carried out with this species and its crossability relations with the common tetraploid potato, *S. tuberosum* L. ssp. *tuberosum*, are unknown. Furthermore, accessions from both countries differed in their morphological phenotypes. To ascertain the feasibility of incorporating this wild germplasm into cultivated potato,  $2n$  pollen screening was carried out in 10 accessions and families derived from crosses between accessions; also, pollen-pistil compatibility relations were studied in reciprocal interspecific crosses. Plants of four of the 10 accessions produced  $2n$  pollen (0.1 to 5.0%) and  $4n$  pollen

(0.0 to 3.0%). Parallel and tripolar spindles at Anaphase II were the cytological mechanisms involved in  $2n$  pollen formation; lack of chromosome migration in both meiotic divisions originated the  $4n$  pollen. Both full compatibility and incompatibility at various sites along the pistil were observed in the *S. tuberosum*  $\times$  *S. okadae* combinations; most reciprocal combinations were incompatible. Compatible genotypes produced  $2n$  pollen. However, only a few seeds were obtained and chromosome counts could not be carried out in the hybrid progeny because seedlings died at an early stage. The pollen-pistil barriers are incomplete and can be circumvented by the appropriate choice of parents. The identification of the postzygotic barriers will be the focus of further studies.

E. L. Camadro (✉) · S. K. Saffarano ·  
J. C. Espinillo · M. Castro  
Laboratorio de Genética, Estación Experimental  
Agropecuaria (EEA) Balcarce, Instituto Nacional de  
Tecnología Agropecuaria (INTA) and Facultad de  
Ciencias Agrarias (FCA), Universidad Nacional de Mar  
del Plata (UNMDP), C.C.276, Balcarce 7620 Bs.As,  
Argentina  
e-mail: ecamadro@balcarce.inta.gov.ar

E. L. Camadro  
Consejo Nacional de Investigaciones Científicas y  
Técnicas (CONICET), Buenos Aires, Argentina

P. W. Simon  
United States Department of Agriculture, Agricultural  
Research Service, 1575 Linden Dr., Madison, WI, USA

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## Introduction

The common potato, *Solanum tuberosum* L. ssp. *tuberosum* (tbr,  $2n = 4x = 48$ , 4EBN), has a narrow genetic base but a large number of closely related wild tuber-bearing species that are important reservoirs of genetic variability for many desirable agronomic, culinary and processing traits (Ross 1976). Most of these species are diploid ( $2n = 2x = 24$ ; 1EBN or 2EBN). They cannot be

directly crossed with tbr when they are compatible at the pollen-pistil level because the hybrid endosperm aborts due to genic unbalance (Johnston et al. 1980). The endosperm barrier can be circumvented if the 2x species produce functional 2n gametes (2n eggs and/or 2n pollen) or if the ploidy level of the common potato is halved by haploidization (Johnston et al. 1980).

*S. okadae* Hawkes et Hjert (oka,  $2n = 2x = 24$ ) is a wild species endemic to Argentina and Bolivia (Hawkes and Hjerting 1983; Ochoa 1990; Spooner and Salas 2006). No genetic studies have been performed with this species and, although it is of potential value in breeding (Hanneman 1996; Pelletier et al. 2001; USDA 2002), its crossability relations with tbr are unknown. Furthermore, accessions from the two countries of origin available at the Potato Germplasm Bank at Balcarce, Argentina, have different morphological phenotypes (Clausen and Ispizúa 2005). In this location, these accessions flower profusely and produce fertile pollen but are difficult to reproduce sexually in controlled crosses (Clausen A.M, pers. comm.). Moreover, its EBN has neither being reported nor deduced from its crossing behavior.

If oka were a 2EBN species, as are most of the wild diploid potatoes, the incorporation of this germplasm into the cultivated pool would be feasible if functional 2n gametes were produced and at least some genotypes were compatible with tbr at the pollen-pistil level. Towards that end, accessions of

oka from Argentina and Bolivia were screened for 2n pollen and crossed to tbr. The results of the screening and the compatibility studies are herein reported, along with the cytological mechanism of 2n pollen formation in the diplandroids that were identified.

## Materials

Six to twenty plants of each of 10 oka accessions or families (Table 1), grown from seeds in a greenhouse, were transplanted into the experimental field in Balcarce along with four tbr cultivars: 'Frital INTA', 'Serrana INTA', 'La Florida' and 'Pampeana INTA', as part of a larger project on field evaluation of wild species. Plants were cultivated from September 2004 until March 2005, when tubers (when present) were harvested. In September 2005, tubers of the few oka plants that tuberized in the field were grown in a greenhouse for further studies. The oka accessions were kindly provided by the Potato Germplasm Bank and the cultivars by the Potato Breeding Program, both belonging to the Estación Experimental Agrop-euaria Balcarce, INTA, Argentina.

## Methods

### 2n pollen screening

In 2004, one or two flowers were collected from individual plants growing in the field. Pollen samples

**Table 1** Geographic location of the *Solanum okadae* accessions used in this study

Accession <sup>1</sup>	Geographic location
Argentina	
<i>OKA 7618 B</i>	Jujuy, Tilcara, Piscuno, 23°36'S 65°11'W
<i>OL 4908</i>	Salta, Chicoana, Quebrada de los Yastos 25°09'S 65°50'W
<i>OKA 7584 A</i>	Jujuy, Capital, Falda de Sombra 24°07'S 65°38'W
<i>OKA 4388 × OKA 4404</i>	Jujuy, Tilcara, Piscuno, 23°38'S 65°06'W; Jujuy, Tilcara, Encrucijada
<i>OKA 4392 B × OKA 4407</i>	Jujuy, Tilcara, Piscuno, 23°34'S 65°17'W; Jujuy, Tilcara, Encrucijada
<i>CLE 1583</i>	Salta, Chicoana, San Martín, 25°10'S 65°49'W
Bolivia	
<i>HPS 2</i>	Bolivia, Larecaja, La Paz, Ilabaya, 17°02'S 67°15'W
<i>HPS 4</i>	Bolivia, Inquisivi, La Paz, 17°02'S 67°15'W
<i>HPS 6</i>	Bolivia, Inquisivi, La Paz, 17°02'S 67° 15' W
<i>HHA 6585 × HPS 6</i>	Bolivia, Ayopaya, Cochabamba, Independencia, 17°06'S 66°55'W; Bolivia, Inquisivi, La Paz, 17°02'S 67° 15'W

<sup>1</sup> Collectors: OKA = Okada KA; OL = Okada KA, Lucarini O; HHR = Hawkes JG, Hjerting JP, Rahn K; CLE = Clausen AM, Erazzú LE

from one or several anthers per plant were placed on glass slides, stained with acetocarmine glycerol jelly and observed under a light microscope. On average, 200 pollen grains per sample were observed in various microscopic fields. The number of large size pollen grains in samples of heterogeneous size, tentatively considered as 2n (Quinn et al. 1974) or 4n, was recorded.

### Meiotic analysis

Immature flower buds of plants that produced 0.1% or more large size pollen were fixed in three parts absolute ethanol: 1 part glacial acetic acid for 24 h and stored in 70% ethanol until used (Mok and Peloquin 1975). With the aid of a needle, meiocytes were removed from individual anthers on a drop of 45% acetocarmine in a glass slide, gently covered with a cover slip and observed under a light microscope.

### Pollen-pistil compatibility analysis

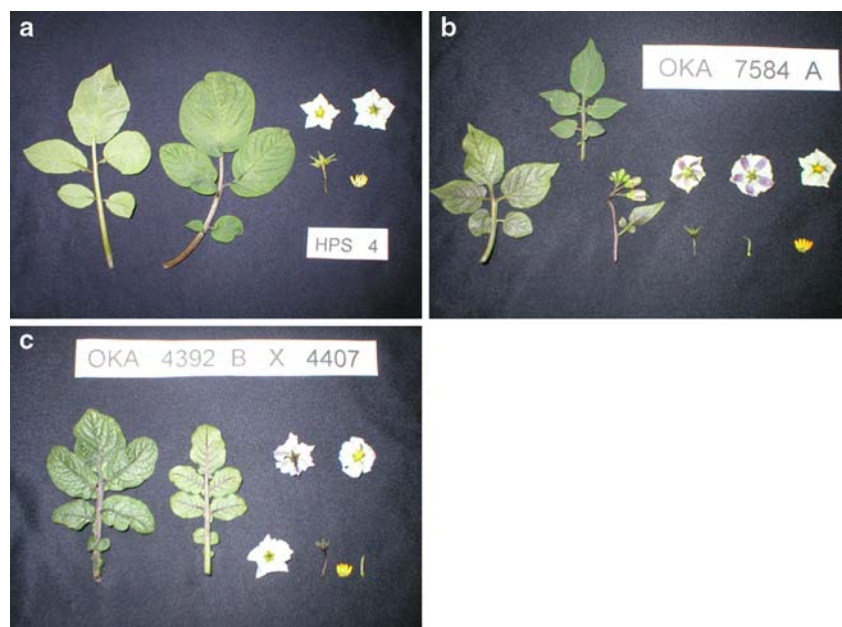
Controlled reciprocal interspecific crosses were made in the field in summer 2004–2005 and in the greenhouse in summer 2005–2006. Three to five flowers were pollinated per genotypic combination (cross). Pollinated styles/pistils were fixed in FAA (8 parts 70% ethanol: 1 part glacial acetic acid: 1 part

40% formaline) 48 h after pollination and stored in the fixative until processed according to Martin (1958). When only styles were fixed, a drop of an aqueous 2,4-D solution (4 ppm) (Dionne 1958) was placed on the remaining ovaries to prevent premature abscission. Fixed styles/pistils were rinsed with tap water and softened with an 8 N NaOH solution for 4 h, rinsed again with tap water and stained with a 0.1% destained aniline blue in 0.1 M  $\text{PO}_4\text{K}_3$  for another 4 h. They were then mounted on a drop of glycerol, squashed with a cover slip and observed under UV light in a microscope.

### Results

Based on plant morphology, each accession was undoubtedly assigned to either one of two well-differentiated phenotypes. All accessions from a given country of origin shared a similar phenotype. The phenotype of the Bolivian accessions (Fig. 1a) closely resembled the holotype specimen of *Solanum okadae* (HAH 6727) published by Hawkes and Hjerting (1989). The phenotype of the Argentinian accessions (Fig. 1b) was similar to the holotype of *S. venatoris* Ochoa (*Ochoa 11917*) published by Ochoa (1990) as *S. okadae*. Families derived from crosses between accessions exhibited segregation for morphological characters (Fig. 1c).

**Fig. 1** Morphology of leaves and reproductive structures of *Solanum okadae*: (a) accession HPS 4 from Bolivia; (b) accession OKA 7584 A from Argentina; (c) hybrid family OKA 4392 B  $\times$  OKA 4407 from Argentina



**Table 2** Number of plants screened for large size pollen, number of plants with 2n pollen and 4n pollen, percentage of monads, dyads and triads in Telophase II, and types of Anaphase II spindle orientation in accessions or families of *Solanum okadae*

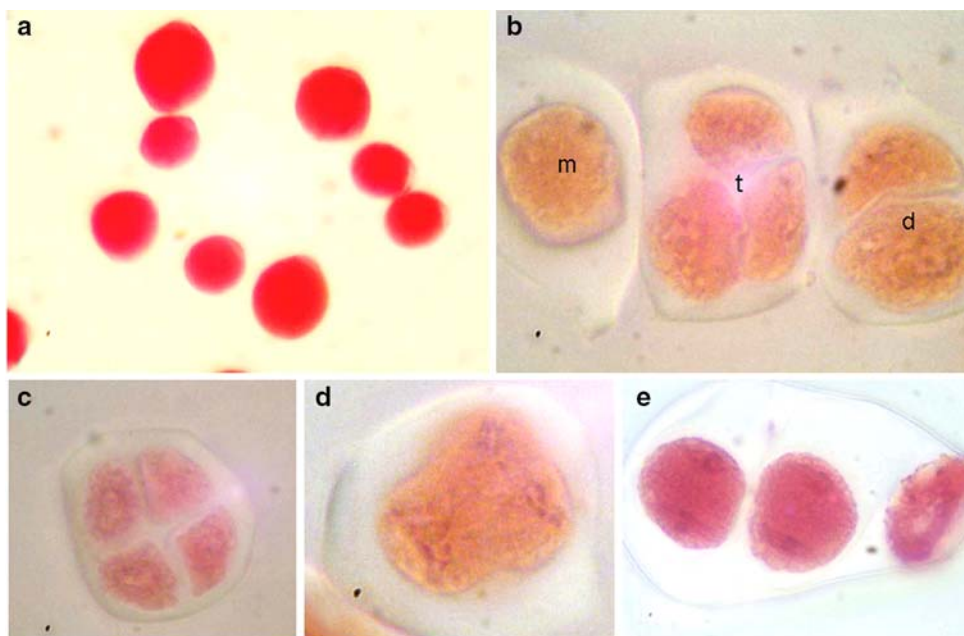
Accession	No. plants	No. plants w/ large pollen	% 2n pollen	% 4n pollen	% monads	% dyads	% triads	Spindle Type <sup>1</sup>
<b>Argentina</b>								
<i>OKA 7618 B</i>	12	4	0.2–1.0	0.0–3.0	0.0–3.0	0.1–0.5	0.0	N
<i>OL 4908</i>	15	0	–	–	–	–	–	N
<i>OKA 7584 A</i>	13	0	–	–	–	–	–	N
<i>OKA 4388</i> × <i>OKA 4404</i>	6	0	–	–	–	–	–	N
<i>OKA 4392 B</i> × <i>OKA 4407</i>	9	0	–	–	–	–	–	N
<i>CLE 1583</i>	8	4	0.5–2.0	0.0–1.5	0.0–2.0	0.5–2.0	0.0	fs
<b>Bolivia</b>								
<i>HPS 2</i>	14	10	0.5–5.0	0.1–2.0	0.1–2.0	0.2–3.0	0.1–1.0	ps, tps, fs
<i>HPS 4</i>	20	0	–	–	–	–	–	N
<i>HPS 6</i>	7	0	–	–	–	–	–	N
<i>HHA 6585</i> × <i>HPS 6</i>	14	6	0.5–5.0	1.0–3.0	1.0–3.0	0.1	0.2	ps, tps

<sup>1</sup> fs = fused; ps = parallel; tps = tripolar, N = normal

## 2n pollen screening

Plants with large size pollen (2n and 4n) were identified in four out of the ten accessions or families derived from crosses between accessions that were screened, both from Argentina and Bolivia

(Table 2). The percentage of plants with 2n pollen varied from 33.3% in accession *OKA 7618 B* from Argentina to 71.4% in accession *HPS 2* from Bolivia. The percentage of 2n pollen in individual plants and accessions varied from 0.1% up to 5.0% and that of 4n pollen (observed in plants that also



**Fig. 2** *Solanum okadae*, accession *CLE 1583* from Argentina: (a) pollen sample of heterogeneous size (400x); meiosis II, tetrad stage: (b) monad (m), dyad (d), triad (t) (1000x) and

(c) tetrad (1000x); anaphase II: (d) tripolar spindles (1000x) and (e) parallel spindles (1000x)

**Table 3** Pollen-pistil compatibility relations in interspecific 4x *Solanum tuberosum* × 2x *S. okadae* crosses carried out in the field

Cross	Pollen-pistil relation
tbr × Argentinian accessions	
‘Frital INTA’ × <i>CLE 1583</i> (1)	C
‘Pampeana INTA’ × <i>CLE 1583</i> (1)	I <sub>2/3</sub>
‘Serrana INTA’ × <i>CLE 1583</i> (1)	C
‘La Florida INTA’ × <i>CLE 1583</i> (1)	I <sub>s</sub> and C
‘Pampeana INTA’ × <i>CLE 1583</i> (3)	C
‘Frital INTA’ × <i>CLE 1583</i> (3)	C
‘Pampeana INTA’ × <i>CLE 1583</i> (5)	I <sub>s</sub>
‘Frital INTA’ × <i>CLE 1583</i> (5)	I <sub>s</sub>
‘Serrana INTA’ × <i>CLE 1583</i> (6)	I <sub>s</sub>
‘Pampeana INTA’ × <i>OKA 7618 B</i> (8)	C
‘La Florida’ × <i>OKA 7618 B</i> (8)	C
‘Serrana INTA’ × <i>OKA 7618 B</i> (8)	C
tbr × Bolivian accessions	
‘Frital INTA’ × ( <i>HHA 6585</i> × <i>HPS 6</i> ) (9)	I <sub>1/3</sub> and I <sub>2/3</sub>
‘Pampeana INTA’ × ( <i>HHA 6585</i> × <i>HPS 6</i> ) (9)	I <sub>1/3</sub>

<sup>1</sup> C = compatible, I<sub>s</sub> = inhibition in stigma, I<sub>1/3</sub> = inhibition in the first third of style; I<sub>2/3</sub> = inhibition in the second third of style

produced 2n pollen) varied between 0 and 5.0% (Fig. 2a).

### Meiotic analysis

Meiosis was studied in plants with large pollen identified in the four accessions previously mentioned, starting the microscopic observations at the latest stages and working backwards. At Telophase II and the tetrad stage, monads and dyads or monads, dyads and triads -in addition to normal tetrads- were observed in these plants (Fig. 2b and c). At Anaphase II, only

normal spindles were observed in *OKA 7618 B* (that had 0.1–0.5% dyads), but only fused spindles were observed in *CLE 1583*, an accession that had a similar percentage of dyads. This type of abnormally oriented spindles was also observed in *HPS 2*, an accession that, as the family *HHA 6585* × *HPS 6*, had both tripolar (Fig. 2d) and parallel spindles (Fig. 2e; Table 2). Lack of chromosome migration in both meiotic divisions was observed in plants with 4n pollen.

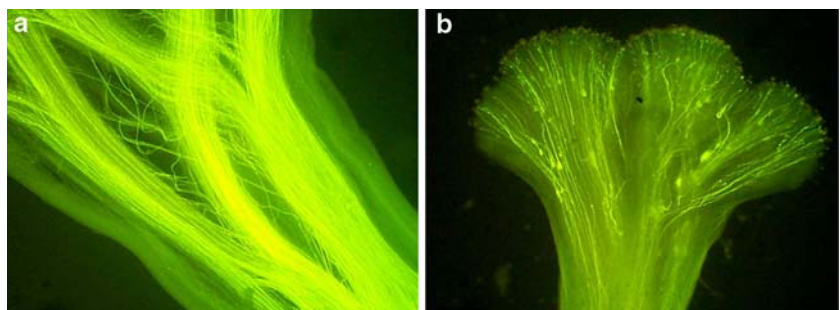
### Pollen-pistil compatibility analysis

Full pollen-pistil compatible relations were observed in eight out of the 14 genotypic tbr × oka combinations carried out in the field (Table 3, Fig. 3a); these compatible combinations involved only accessions from Argentina. Various inhibition sites were observed in the remaining combinations: only in the stigma (three combinations, not shown), only in the first third of the style (Fig. 3b) or the second third of the style or in both (one combination each, not shown). One plant derived from a cross between Bolivian accessions -(*HHA 6585* × *HPS 6*) (9)-exhibited incompatible relations with two different tbr cultivars. The three genotypes that exhibited compatibility with tbr, *CLE 1583* (1), *CLE 1583* (3) and *OKA 7618 B* (8), had been previously identified as 2n pollen producers by cytological observations of pollen samples and meiotic studies (Table 2).

In contrast with the field results, all the tbr × oka genotypic combinations carried out in the greenhouse were incompatible. Similar incompatibility sites were observed in both studies (Table 4).

In the reciprocal direction of the cross (oka × tbr), carried out in the greenhouse, only two genotypic combinations, *OKA 7584* (3) × ‘Pampeana INTA’ and (*OKA 4392 B* × *OKA 4407*) (1) × ‘Frital INTA’, were compatible (Table 5).

**Fig. 3** Pollen-pistil relations in genotypic *Solanum okadae*-*S. tuberosum* combinations: (a) fully compatible (1000×); (b) incompatibility in first third of the style (400×)





**Table 4** Pollen-pistil compatibility relations in interspecific 4x *Solanum tuberosum* × 2x *S. okadae* crosses carried out in the greenhouse

Cross	Pollen-pistil relations
tbr × Argentinian accessions	
‘Pampeana INTA’ × <i>OKA 7584</i> (3)	I <sub>s</sub> (in a few, I <sub>1/3</sub> )
‘Pampeana INTA’ × <i>OKA 7584</i> (13)	I <sub>2/3</sub>
tbr × Bolivian accessions	
‘Frital INTA’ × <i>HPS 2</i> (1B)	I <sub>s</sub> , I <sub>1/3</sub>
‘Frital INTA’ × <i>HPS 2</i> (1)	I <sub>s</sub>
‘Pampeana INTA’ × <i>HPS 2</i> (1)	I <sub>s</sub>
‘Serrana INTA’ × <i>HPS 2</i> (1)	I <sub>s</sub> (in a few, I <sub>1/3</sub> )
‘Frital INTA’ × ( <i>HHA 6585</i> × <i>HPS 6</i> ) (1)	I <sub>s</sub>
‘Pampeana INTA’ × ( <i>HHA 6585</i> × <i>HPS 6</i> ) (1)	I <sub>s</sub>
‘Serrana INTA’ × ( <i>HHA 6585</i> × <i>HPS 6</i> ) (1)	I <sub>s</sub>

<sup>1</sup> C = compatible, I<sub>s</sub> = inhibition in stigma, I<sub>1/3</sub> = inhibition in first third of style; I<sub>2/3</sub> = inhibition in the second third of style

Fruits were obtained in the greenhouse from three genotypic combinations: *HPS 2* (1) × ‘Frital INTA’, (*OKA 4392 B* × *OKA 4407*) (1) × ‘Frital INTA’ and *OKA 7584* (3) × ‘Pampeana INTA’. These fruits had, respectively, none, three and 13 small viable seeds. These seeds germinated but the plantlets derived from them died at an early developmental stage. Thus, chromosome counts could not be carried out.

## Discussion

The *S. okadae* accessions had to be grown in a greenhouse to be used as female parents due to the difficulties encountered when they were grown in the field. Since there are no reports in the potato literature on genotype × environment interactions affecting interspecific pollen-pistil compatibility relations, this fact is not expected to affect our conclusions.

Accessions of *S. okadae* from Argentina and Bolivia could be clearly distinguished by their plant morphological phenotypes that closely resembled, respectively, the holotype specimen of *S. venatoris* Ochoa published by Ochoa (1990) and the holotype specimen of *S. okadae* published by Hawkes and Hjerting (1989). Clausen and Ispizúa (2005) recently reported notable morphological differences between

**Table 5** Pollen-pistil compatibility relations in interspecific 2x *Solanum okadae* × 4x *S. tuberosum* crosses carried out in the greenhouse

Cross	Pollen-pistil relation
Argentinian accessions × tbr	
<i>OL 4908</i> (1) × ‘Pampeana INTA’	I <sub>s</sub>
<i>OL 4908</i> (1) × ‘Frital INTA’	I <sub>s</sub>
<i>OKA 7584</i> (2) × ‘Pampeana INTA’	I <sub>s</sub> (in a few, I <sub>1/3</sub> )
<i>OKA 7584</i> (3) × ‘Pampeana INTA’	C
<i>OKA 7584</i> (6) × ‘Pampeana INTA’	I <sub>s</sub>
( <i>OKA 4392 B</i> × <i>OKA 4407</i> ) (1) × ‘Frital INTA’	C
( <i>HHA 6585</i> × <i>HPS 6</i> ) (1) × ‘Frital INTA’	I <sub>s</sub>
( <i>HHA 6585</i> × <i>HPS 6</i> ) (1) × ‘Pampeana INTA’	I <sub>2/3</sub> (in a few, I <sub>1/3</sub> )
Bolivian accessions × tbr	
<i>HPS 2</i> (1) × ‘Frital INTA’	I <sub>s</sub>
<i>HPS 2</i> (1) × ‘Serrana INTA’	I <sub>1/3</sub>
<i>HPS 6</i> (4) × ‘Frital INTA’	I <sub>1/3</sub>

<sup>1</sup> C = compatible, I<sub>s</sub> = inhibition in stigma, I<sub>1/3</sub> = inhibition in first third of style; I<sub>2/3</sub> = inhibition in the second third of style

accessions from both countries but did not speculate on the possible causes. Similar numbers of plants of each origin were crossed as male parents to tbr. Although the number of genotypic combinations analyzed was not high (22), accessions from both countries differed in their compatibility relations with the cultivated potato. This observation, in addition to the distinct geographic distribution and morphological differences observed, highlight the need to reconsider their classification as a single species.

The presence of monads, dyads and triads along with normal tetrads at Telophase II in accessions with pollen of heterogeneous size gave support to the presumption that large size pollen was 2n and the largest class was 4n. The cytological mechanisms leading to 2n pollen formation in these accessions were parallel and fused spindles (that gave rise to dyads) and tripolar spindles (that gave rise to triads). These three types of spindle orientation originate FDR 2n pollen, giving further support to the assertion that genetically controlled 2n gametes have been instrumental in the evolution of the tuber-bearing *Solanum* (den Nijs and Peloquin 1977).

The compatible pollen-pistil relations observed are an indication that the pre-zygotic barriers between

oka and tbr are incomplete and can, likely, be circumvented by the appropriate choice of parents. In addition, the oka genotypes identified as compatible with tbr -OKA 7618 B (8) and CLE 1583 (1) and (3)-produced 2n pollen and also 4n pollen in relatively high frequencies. However, only a few seeds were obtained from these crosses. Since chromosome numbers could not be determined due to early plantlet death, an EBN cannot be assigned to the species as a result of this study. Not having been established if the post-zygotic failure resides in the endosperm or in the embryo itself, strategies to circumvent the post-zygotic barriers cannot be proposed at this time. The identification of the post-zygotic barriers will be the focus of further studies.

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